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(54) A TNF homologue, TL5

(57) TL5 polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing TL5 polypeptides and polynucleotides in the design of protocols for the treatment of chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g., inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g., lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, among others, and diagnostic assays for such conditions.

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Description

This application claims the benefit of U.S. Provisional Application No: 60/041,797, filed April 2, 1997.

FIELD OF INVENTION

This invention relates to newly identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to TNF family, hereinafter referred to as TL5. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides.

BACKGROUND OF THE INVENTION

Many biological actions, for instance, response to certain stimuli and natural biological processes, are controlled by factors, such as cytokines. Many cytokines act through receptors by engaging the receptor and producing an intracellular response.

For example, tumor necrosis factors (TNF) alpha and beta are cytokines which act through TNF receptors to regulate numerous biological processes, including protection against infection and induction of shock and inflammatory disease. The TNF molecules belong to the "TNF-ligand" superfamily, and act together with their receptors or counter-ligands, the "TNF-receptor" superfamily. So far, nine members of the TNF ligand superfamily have been identified and ten members of the TNF-receptor superfamily have been characterized.

Among the ligands there are included are TNF-α, lymphotoxin-α (LT-α, also known as TNF-β), LT-β (found in complex heterotrimer LT-α2-β), FasL, CD40L, CD27L, CD30L, 4-1BBL, OX40L and TRAIL. The superfamily of TNF receptors includes the p55TNF receptor, p75TNF receptor, TNF receptor-related protein, FAS antigen or APO-1, CD40, CD27, CD30, 4-1BB, OX40, low affinity p75 and NGF-receptor (Meager, A., Biologicals, 22:291-295(1994)).

Many members of the TNF-ligand superfamily are expressed by activated T-cells, implying that they are necessary for T-cell interactions with other cell types which underlie cell ontogeny and functions. (Meager, A., supra).

Considerable insight into the essential functions of several members of the TNF family has been gained from the identification and creation of mutants that abolish the expression of these proteins. For example, naturally occurring mutations in the FAS antigen and its ligand cause lymphoproliferative disease (Watanabe-Fukunaga, R., et al., Nature 356:314 (1992)), perhaps reflecting a failure of programmed cell death. Mutations of the CD40 ligand cause an X-linked immunodeficiency state characterized by high levels of immunoglubulin M and low levels of immunoglobulin G in plasma, indicating faulty T-cell-dependent B-cell activation (Allen, R.C. et al., Science 259:990 (1993)). Targeted mutations of the low affinity nerve growth factor receptor cause a disorder characterized by faulty sensory innovation of peripheral structures (Lee, K.F. et al, Cell 69:737 (1992)).

TNF and LT-α are capable of binding to two TNF receptors (the 55- and 75-kd TNF receptors). A large number of biological effects elicited by TNF and LT-α, acting through their receptors, include hemorrhagic necrosis of transplanted tumors, cytotoxicity, a role in endotoxic shock, inflammation, immunoregulation, proliferation and anti-viral responses, as well as protection against the deleterious effects of ionizing radiation. TNF and LT-α are involved in the pathogenesis of a wide range of diseases, including endotoxic shock, cerebral malaria, tumors, autoimmuine disease, AIDS and graft-host rejection (Beutler, B. and Von Huffel, C., Science 264:667-668 (1994)). Mutations in the p55 Receptor cause increased susceptibility to microbial infection.

Moreover, an about 80 amino acid domain near the C-terminus of TNFR1 (P55) and Fas was reported as the "death domain," which is responsible for transducing signals for programmed cell death (Tartaglia et al., Cell 74:845 (1993)).

The effects of TNF family ligands and TNF family receptors are varied and influence numerous functions, both normal and abnormal, in the biological processes of the mammalian system. There is a clear need, therefore, for identification and characterization of such receptors and ligands that influence biological activity, both normally and in disease states. In particular, there is a need to isolate and characterize novel members of the TNF family.

This indicates that the TNF family has an established, proven history as therapeutic targets. Clearly there is a need for identification and characterization of further members of TNF family which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g., inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g., lymphoproliferative disorders), atherosclerosis, and Alzheimers disease.

SUMMARY OF THE INVENTION

In one aspect, the invention relates to TL5 polypeptides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such TL5 polypeptides and polynucleotides. Such uses include the treatment of chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g., inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g., lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, among others. In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with TL5 imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate TL5 activity or levels.

DESCRIPTION OF THE INVENTION

Definitions

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"TL5" refers, among others, generally to a polypeptide having the amino acid sequence set forth in SEQ ID NO:2 or an allelic variant thereof.

"TL5 activity or TL5 polypeptide activity" or "biological activity of the TL5 or TL5 polypeptide" refers to the metabolic or physiologic function of said TL5 including similar activities or improved activities or these activities with decreased undesirable side-effects. Also included are antigenic and immunogenic activities of said TL5.

"TL5 gene" refers to a polynucleotide having the nucleotide sequence set forth in SEQ ID NO: 1 or allelic variants thereof and/or their complements.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of an Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from the natural state. If an "isolated" composition or substance occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single-and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to both short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor forma-

tion, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter *et al.*, "Analysis for protein modifications and nonprotein cofactors", *Meth Enzymol* (1990) 182:626-646 and Rattan *et al.*, "Protein Synthesis: Posttranslational Modifications and Aging", *Ann NY Acad Sci* (1992) 663:48-62.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" *per se* has an art-recognized meaning and can be calculated using published techniques. See, e.g.: (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed., Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., *SIAM J Applied Math* (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCS program package (Devereux, J., *et al., Nucleic Acids Research* (1984) 12(1):387), BLASTP, BLASTN, FASTA (Atschul, S.F. *et al., J Molec Biol* (1990) 215:403).

As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence of SEQ ID NO: 1 is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence of SEQ ID NO: 1. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5 or 3 terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

Similarly, by a polypeptide having an amino acid sequence having at least, for example, 95% "identity" to a reference amino acid sequence of SEQ ID NO:2 is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of SEQ ID NO: 2. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

Polypeptides of the Invention

In one aspect, the present invention relates to TL5 polypeptides (or TL5 proteins). The TL5 polypeptides include the polypeptide of SEQ ID NOS:2 and 4; as well as polypeptides comprising the amino acid sequence of SEQ ID NO: 2; and polypeptides comprising the amino acid sequence which have at least 80% identity to that of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2. Furthermore, those with at least 97-99% are highly preferred. Also included within TL5 polypeptides are polypeptides having the amino acid sequence which have at least 80% identity to the polypeptide having the amino acid sequence of SEQ ID NO:2 over its entire length, and still more preferably at least 90% identity, and still more preferably at least 95% identity to SEQ ID NO:2. Furthermore, those with at least 97-99% are highly preferred. Preferably TL5 polypeptide exhibit at least one biological activity of TL5.

The TL5 polypeptides may be in the form of the "mature" protein or may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production.

Fragments of the TL5 polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that entirely is the same as part, but not all, of the amino acid sequence of the aforementioned TL5 polypeptides. As with TL5 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, and 101 to the end of TL5 polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of TL5 polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments. Biologically active fragments are those that mediate TL5 activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

Preferably, all of these polypeptide fragments retain the biological activity of the TL5, including antigenic activity. Among the most preferred fragment is that having the amino acid sequence of SEQ ID NO: 4. Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions -- i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

The TL5 polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

Polynucleotides of the Invention

Another aspect of the invention relates to TL5 polynucleotides. TL5 polynucleotides include isolated polynucleotides which encode the TL5 polypeptides and fragments, and polynucleotides closely related thereto. More specifically, TL5 polynucleotide of the invention include a polynucleotide comprising the nucleotide sequence contained in SEQ ID NO: 1 encoding a TL5 polypeptide of SEQ ID NO: 2, and polynucleotides having the particular sequences of SEQ ID NOS:1 and 3. TL5 polynucleotides further include a polynucleotide comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the TL5 polypeptide of SEQ ID NO:2, and a polynucleotide comprising a nucleotide sequence that is at least 80% identical to that of SEQ ID NO:1 over its entire length. In this regard, polynucleotides at least 90% identical are particularly preferred, and those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred. Also included under TL5 polynucleotides are a

nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO:1 to hybridize under conditions useable for amplification or for use as a probe or marker. The invention also provides polynucleotides which are complementary to such TL5 polynucleotides.

TL5 of the invention is structurally related to other proteins of the TNF family, as shown by the results of sequencing the cDNA encoding human TL5. The cDNA sequence of SEQ ID NO:1 contains an open reading frame (nucleotide number 154 to 1008) encoding a polypeptide of 285 amino acids of SEQ ID NO:2. The amino acid sequence of Table 1 (SEQ ID NO:2) has about 34% identity (using BLAST) in 35 amino acid residues with Canis familiaris TNF. Swissprot accession no. P51742/TNFA_CANFA. The nucleotide sequence of Table 1 (SEQ ID NO:1) has about 99% identity (using BLAST) in 376 nucleotide residues with Homo sapiens cDNA clone 593690 3' (Genbank Accession No. AA166695). Furthermore, TL5 (SEQ ID NO:1) is 97% identical to human STS SHGC-36171 over 290 nucleotide base residues (Genbank Accesssion No. G30081). Thus, TL5 polypeptides and polynucleotides of the present invention are expected to have, inter alia, similar biological functions/properties to their homologous polypeptides and polynucleotides, and their utility is obvious to anyone skilled in the art.

Table 1[a]

```
   1  CACGAGAAAA TTCAGGATAA CTCTCCTGAG GGGTGAGCCA AGCCCTGCCA

  51  TGTAGTGCAC GCAGGACATC AACAAACACA GATAACAGGA AATGATCCAT

 101  TCCCTGTGGT CACTTATTCT AAAGGCCCCA ACCTTCAAAG TTCAAGTAGT

 151  GATATGGATG ACTCCACAGA AAGGGAGCAG TCACGCCTTA CTTCTTGCCT

 201  TAAGAAAAGA GAAGAAATGA AACTGAAGGA GTGTGTTTCC ATCCTCCCAC

 251  GGAAGGAAAG CCCCTCTGTC CGATCCTCCA AAGACGGAAA GCTGCTGGCT

 301  GCAACCTTGC TGCTGGCACT GCTGTCTTGC TGCCTCACGG TGGTGTCTTT

 351  CTACCAGGTG GCCGCCCTGC AAGGGGACCT GGCCAGCCTC CGGGCAGAGC

 401  TGCAGGGCCA CCACGCGGAG AAGCTGCCAG CAGGAGCAGG AGCCCCCAAG
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 451  GCCGGCCTGG AGGAAGCTCC AGCTGTCACC GCGGGACTGA AAATCTTTGA
 501  ACCACCAGCT CCAGGAGAAG GCAACTCCAG TCAGAACAGC AGAAATAAGC
 551  GTGCCGTTCA GGGTCCAGAA GAAACAGTCA CTCAAGACTG CTTGCAACTG
 601  ATTGCAGACA GTGAAACACC AACTATACAA AAAGGATCTT ACACATTTGT
 651  TCCATGGCTT CTCAGCTTTA AAAGGGGAAG TGCCCTAGAA GAAAAAGAGA
 701  ATAAAATATT GGTCAAAGAA ACTGGTTACT TTTTTATATA TGGTCAgGTT
 751  TTATATACTG ATAAGACCTA CGCCATGGGA CATCTAATTC AGAGGAAGAA
 801  GGTCCATGTC TTTGGGGATG AATTGAGTCT GGTGACTTTG TTTCGATGTA
 851  TTCAAAATAT GCCTGAAACA CTACCCAATA ATTCCTGCTA TTCAGCTGGC
 901  ATTGCAAAAC TGGAAGAAGG AGATGAACTC CAACTTGCAA TACCAAGAGA
 951  AAATGCACAA ATATCACTGG ATGGAGATGT CACATTTTTT GGTGCATTGA
1001  AACTGCTGTG ACCTACTTAC ACCATGTCTG TAGCTATTTT CCTCCCTTTC
1051  TCTGTACCTC TAAGAAGAAA GAATCTAACT GAAAATACCA AAA
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[a] A nucleotide sequence of a human TL5 (SEQ ID NO: 1).

Table 2[b]

```
  1  MDDSTEREQS RLTSCLKKRE EMKLKECVSI LPRKESPSVR SSKDGKLLAA

 51  TLLLALLSCC LTVVSFYQVA ALQGDLASLR AELQGHHAEK LPAGAGAPKA

101  GLEEAPAVTA GLKIFEPPAP GEGNSSQNSR NKRAVQGPEE TVTQDCLQLI

151  ADSETPTIQK GSYTFVPWLL SFKRGSALEE KENKILVKET GYFFIYGQVL

201  YTDKTYAMGH LIQRKKVHVF GDELSLVTLF RCIQNMPETL PNNSCYSAGI

251  AKLEEGDELQ LAIPRENAQI SLDGDVTFFG ALKLL
```

[b] An amino acid sequence of a human TL5 (SEQ ID NO: 2).

One polynucleotide of the present invention encoding TL5 may be obtained using standard cloning and screening, from a cDNA library derived from mRNA in cells of human Fetal liver spleen, chronic lymphocytic leukemia spleen, ovarian cancer, stomach cancer, smooth muscle cells, neutrophils, PMA stimulated T cells, oxidized LDL stimulated macrophages, dendritic cells, bone marrow cells and cell lines, and CD34+cord blood using the expressed sequence tag (EST) analysis (Adams, M.D., *et al. Science* (1991) 252:1651-1656; Adams, M.D. *et al., Nature,* (1992) *355:* 632-634; Adams, M.D., *et al., Nature* (1995) 377 Supp:3-174). Polynucleotides of the invention can also be obtained from natural sources such as genomic DNA libraries or can be synthesized using well known and commercially available techniques.

The nucleotide sequence encoding TL5 polypeptide of SEQ ID NO:2 may be identical to the polypeptide encoding sequence contained in Table 1 (nucleotide number 154 to 1008 of SEQ ID NO:1), or it may be a sequence, which as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2.

When the polynucleotides of the invention are used for the recombinant production of TL5 polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz *et al., Proc Natl Acad Sci USA* (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further preferred embodiments are polynucleotides encoding TL5 variants comprising the amino acid sequence of TL5 polypeptide of Table 2 (SEQ ID NO:2) in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination. Among the preferred polynucleotides of the present invention is contained in Table 3 (SEQ ID NO: 3) encoding the amino acid sequence of Table 4 (SEQ ID NO: 4).

Table 3[c]

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  1  GGGAGAAGGC AACTCCAGTC AGAACAGCAG AAATAAGCGT GCCGTTCAGG
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```
 51  GTCCAGAAGA AACAGGATCT TACGAGACAT TTGTTCCATG GCTTCTCAGC

101  TTTAAAAGGG GAAGTGCCCT AGAAGAAAAA GAGAATAAAA TATTGGTCAA

151  AGAAACTGGT TACTTTTTTA TATATGGTCA GGTTTTATAT ACTGATAAGA

201  CCTACGCCAT GGGACATCTA ATTCAGAGGA AGAAGGTCCA TGTCTTTGGG

251  GATGAATTGA GTCTGGTGAC TTTGTTTCGA TGTATTCAAA ATATGCCTGA

301  AACACTACCC AATAATTCCT GCTATTCAGC TGGCATTGCA AAACTGGAAG

351  AAGGAGATGA ACTCCAACTT GCAATACCAA GAGAAAATGC ACAAATATCA

401  CTGGATGGAG ATGTCACATT TTTTGGTGCA TTGAAACTGC TGTGACCTAC

451  TTACACCATG TCTGTAGCTA TTTTCCTCCC TTTCTCTGTA CCTCTAAGAA

501  GAAAGAATCT AACTGAAAAT ACCAAAAAAA
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[c] A partial nucleotide sequence of a human TL5 (SEQ ID NO: 3).

Table 4[d]

```
  1  GEGNSSQNSR NKRAVQGPEE TGSYETFVPW LLSFKRGSAL EEKENKILVK

 51  ETGYFFIYGQ VLYTDKTYAM GHLIQRKKVH VFGDELSLVT LFRCIQNMPE

101  TLPNNSCYSA GIAKLEEGDE LQLAIPRENA QISLDGDVTF FGALKLL
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[d] A partial amino acid sequence of a human TL5 (SEQ ID NO: 4).

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the herein above-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 80%, and preferably at least 90%, and more preferably at least 95%, yet even more preferably 97-99% identity between the sequences.

Polynucleotides of the invention, which are identical or sufficiently identical to a nucleotide sequence contained in SEQ ID NO:1 or a fragment thereof (including that of SEQ ID NO:3), may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding TL5 polypeptide and to isolate cDNA and genomic clones of other genes (including genes encoding homologs and orthologs from species other than human) that have a high sequence similarity to the TL5 gene. Such hybridization techniques are known to those of skill in the art. Typically these nucleotide sequences are 80% identical, preferably 90% identical, more preferably 95% identical

to that of the referent. The probes generally will comprise at least 15 nucleotides. Preferably, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

In one embodiment, to obtain a polynucleotide encoding TL5 polypeptide, including homologs and orthologs from species other than human, comprises the steps of screening an appropriate library under stingent hybridization conditions with a labeled probe having the SEQ ID NO: 1 or a fragment thereof (including that of SEQ ID NO: 3), and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to those of skill in the art. Thus in another aspect, TL5 polynucleotides of the present invention further include a nucleotide sequence comprising a nucleotide sequence that hybridize under stringent condition to a nucleotide sequence having SEQ ID NO: 1 or a fragment thereof (including that of SEQ ID NO:3). Also included with TL5 polypeptides are polypeptide comprising amino acid sequence encoded by nucleotide sequence obtained by the above hybridization condition. Stringent hybridization conditions are as defined above or, alternatively, conditions under overnight incubation at 42°C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate, and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65°C.

The polynucleotides and polypeptides of the present invention may be employed as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

Vectors, Host Cells, Expression

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., *BASIC METHODS IN MOLECULAR BIOLOGY* (1986) and Sambrook et al., *MOLECULAR CLONING: A LABORATORY MANUAL,* 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, *E. coli, Streptomyces* and *Bacillus subtilis* cells; fungal cells, such as yeast cells and *Aspergillus* cells; insect cells such as *Drosophila* S2 and *Spodoptera* Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al., MOLECULAR CLONING, A LABORATORY MANUAL (supra).*

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

If the TL5 polypeptide is to be expressed for use in screening assays, generally, it is preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. If TL5 polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered. TL5 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

This invention also relates to the use of TL5 polynucleotides for use as diagnostic reagents. Detection of a mutated form of TL5 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of TL5. Individuals carrying mutations in the TL5 gene may be detected at the DNA level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled TL5 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers *et al., Science* (1985) 230: 1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton *et al., Proc Natl Acad Sci USA* (1985) 85: 4397-4401. In another embodiment, an array of oligonucleotides probes comprising TL5 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee *et al., Science,* Vol 274, pp 610-613 (1996)).

The diagnostic assays offer a process for diagnosing or determining a susceptibility to chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g., inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g., lymphoproliferative disorders), atherosclerosis, and Alzheimers disease through detection of mutation in the TL5 gene by the methods described.

In addition, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g., inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g., lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of TL5 polypeptide or TL5 mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an TL5 polypeptide, in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

Thus in another aspect, the present invention relates to a diagonostic kit for a disease or suspectability to a disease, particularly chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g., inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g., lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, which comprises:

(a) a TL5 polynucleotide, preferably the nucleotide sequence of SEQ ID NO: 1, or a fragment thereof;
(b) a nucleotide sequence complementary to that of (a);
(c) a TL5 polypeptide, preferably the polypeptide of SEQ ID NO: 2, or a fragment thereof; or
(d) an antibody to a TL5 polypeptide, preferably to the polypeptide of SEQ ID NO: 2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

Chromosome Assays

The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal

region are then identified through linkage analysis (coinheritance of physically adjacent genes). The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

The gene for TL5 was localized to chromosome 13.

Antibodies

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them can also be used as immunogens to produce antibodies immunospecific for the TL5 polypeptides. The term "immunospecific" means that the antibodies have substantiall greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against the TL5 polypeptides can be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., *Nature* (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor *et al., Immunology Today* (1983) 4:72) and the EBV-hybridoma technique (Cole *et al.,* MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can also be adapted to produce single chain antibodies to polypeptides of this invention. Also, transgenic mice, or other organisms including other mammals, may be used to express humanized antibodies.

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against TL5 polypeptides may also be employed to treat chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g., inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g., lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, among others.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with TL5 polypeptide, or a fragment thereof, adequate to produce antibody and/or T cell immune response to protect said animal from chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g., inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g., lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, among others. Yet another aspect of the invention relates to a method of inducing immunological response in a mammal which comprises, delivering TL5 polypeptide via a vector directing expression of TL5 polynucleotide *in vivo* in order to induce such an immunological response to produce antibody to protect said animal from diseases.

Further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to a TL5 polypeptide wherein the composition comprises a TL5 polypeptide or TL5 gene. The vaccine formulation may further comprise a suitable carrier. Since TL5 polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation instonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Screening Assays

The TL5 polypeptide of the present invention may be employed in a screening process for compounds which stimulate (agonists) or inhibit (antagonists, or otherwise called inhibitors) the binding, synthesis or action of the TL5 polypeptide of the present invention. Thus, polypeptides of the invention may also be used to assess or identify agonist

or antagonists from, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These agonists or antagonists may be natural substrates, ligands, receptors, etc., as the case may be, of the polypeptide of the present invention; or may be structural or functional mimetics of the polypeptide of the present invention. See Coligan *et al., Current Protocols in Immunology* 1(2):Chapter 5 (1991).

TL5 polypeptides are responsible for many biological functions, including many pathologies. Accordingly, it is desirous to find compounds and drugs which stimulate TL5 polypeptide on the one hand and which can inhibit the function of TL5 polypeptide on the other hand. In general, agonists are employed for therapeutic and prophylactic purposes for such conditions as chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g., inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g., lymphoproliferative disorders), atherosclerosis, and Alzheimers disease. Antagonists may be employed for a variety of therapeutic and prophylactic purposes for such conditions as chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g., inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g., lymphoproliferative disorders), atherosclerosis, and Alzheimers disease.

In general, such screening procedures may involve using appropriate cells which express the TL5 polypeptide or respond to TL5 polypeptide of the present invention. Such cells include cells from mammals, yeast, *Drosophila* or *E. coli*. Cells which express the TL5 polypeptide (or cell membrane containing the expressed polypeptide) or respond to TL5 polypeptide are then contacted with a test compound to observe binding, or stimulation or inhibition of a functional response. The ability of the cells which were contacted with the candidate compounds is compared with the same cells which were not contacted for TL5 activity.

The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the TL5 polypeptide is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor. Further, these assays may test whether the candidate compound results in a signal generated by activation of the TL5 polypeptide, using detection systems appropriate to the cells bearing the TL5 polypeptide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed.

Alternatively, TL5 may be expressed as a soluble protein, including versions which fuse all or part of TL5 with a convenient partner peptide for which detection reagents are available, eg TL5-IgG fusions, and used in a solid state or solution phase binding assay. For example, the soluble TL5 can be used to detect agonist or antagonist binding directly through changes that can be detected experimentally, eg surface plasmon resonance, nuclear magnetic resonance spectrometry, sedimentation, calorimetry. The soluble TL5 can be used to detect agonist or antagonist binding directly by looking for competition of the candidate agonist or antagonist with a receptor whose binding can be detected. Receptor detection methods include antibody recognition, modification of the receptor via radioactive labeling, chemical modification (e.g., biotinylation), fusion to an epitope tag. Methods include ELISA based assays, immunoprecipitation and scintillation proximity. The receptor may also be obtained from natural sources (e.g., cells, cell membranes and cell supernatnants), but in these cases one would prefer to detect the binding of TL5 through methods including antibody recognition, modification of TL5 via radioactive labeling, chemical modification of TL5 (e.g., biotinylation), or fusion of TL5 to an epitope tag.

The TL5 cDNA, protein and antibodies to the protein may also be used to configure assays for detecting the effect of added compounds on the production of TL5 mRNA and protein in cells. For example, an ELISA may be constructed for measuring secreted or cell associated levels of TL5 protein using monoclonal and polyclonal antibodies by standard methods known in the art, and this can be used to discover agents which may inhibit or enhance the production of TL5 (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

The TL5 protein may be used to identify membrane bound or soluble receptors, if any, through standard receptor binding techniques known in the art. These include, but are not limited to, ligand binding and crosslinking assays in which the TL5 is labeled with a radioactive isotope (e.g., 125I), chemically modified (e.g., biotinylated), or fused to a peptide sequence suitable for detection or purification, and incubated with a source of the putative receptor (cells, cell membranes, cell supernatants, tissue extracts, bodily fluids). Other methods include biophysical techniques such as surface plasmon resonance and spectroscopy. In addition to being used for purification and cloning of the receptor, these binding assays can be used to identify agonists and antagonists of TL5 which compete with the binding of TL5 to its receptors, if any. Standard methods for conducting screening assays are well understood in the art.

Examples of potential TL5 polypeptide antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligands, substrates, enzymes, receptors, etc., as the case may be, of the TL5 polypeptide, e.g., a fragment of the ligands, substrates, enzymes, receptors, etc.; or small molecules which bind to the polypeptide of the present invention but do not elicit a response, so that the activity of the polypeptide is prevented.

Thus in another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, etc. for TL5 polypeptides; or compounds which decrease or enhance the production

of TL5 polypeptides, which comprises:

(a) a TL5 polypeptide, preferably that of SEQ ID NO:2;
(b) a recombinant cell expressing a TL5 polypeptide, preferably that of SEQ ID NO:2;
(c) a cell membrane expressing a TL5 polypeptide; preferably that of SEQ ID NO: 2; or
(d) antibody to a TL5 polypeptide, preferably that of SEQ ID NO: 2.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

Prophylactic and Therapeutic Methods

This invention provides methods of treating abnormal conditions such as, chronic and acute inflammation, arthritis, septicemia, autoimmune diseases (e.g., inflammatory bowel disease, psoriasis), transplant rejection, graft vs. host disease, infection, stroke, ischemia, acute respiratory disease syndrome, restenosis, brain injury, AIDS, Bone diseases, cancer (e.g., lymphoproliferative disorders), atherosclerosis, and Alzheimers disease, related to both an excess of and insufficient amounts of TL5 polypeptide activity.

If the activity of TL5 polypeptide is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit the function of the TL5 polypeptide, such as, for example, by blocking the binding of ligands, substrates, enzymes, receptors, etc., or by inhibiting a second signal, and thereby alleviating the abnormal condition. In another approach, soluble forms of TL5 polypeptides still capable of binding the ligand, substrate, enzymes, receptors, etc. in competition with endogenous TL5 polypeptide may be administered. Typical embodiments of such competitors comprise fragments of the TL5 polypeptide.

In another approach, soluble forms of TL5 polypeptides still capable of binding the ligand in competition with endogenous TL5 polypeptide may be administered. Typical embodiments of such competitors comprise fragments of the TL5 polypeptide.

In still another approach, expression of the gene encoding endogenous TL5 polypeptide can be inhibited using expression blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, *J Neurochem* (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Alternatively, oligonucleotides which form triple helices with the gene can be supplied. See, for example, Lee *et al., Nucleic Acids Res* (1979) 6:3073; Cooney *et al., Science* (1988) 241:456; Dervan *et al., Science* (1991) 251:1360. These oligomers can be administered *per se* or the relevant oligomers can be expressed *in vivo*.

For treating abnormal conditions related to an under-expression of TL5 and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates TL5 polypeptide, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of TL5 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells *in vivo* and expression of the polypeptide *in vivo*. For overview of gene therapy, see Chapter 20, *Gene Therapy and other Molecular Genetic-based Therapeutic Approaches*, (and references cited therein) in Human Molecular Genetics, T Strachan and A P Read, BIOS Scientific Publishers Ltd (1996). Another approach is to administer a therapeutic amount of TL5 polypeptides in combination with a suitable pharmaceutical carrier.

Formulation and Administration

Peptides, such as the soluble form of TL5 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Such carriers include but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels and the like.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 μg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide *ex vivo,* and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

Example 1

An EST (EST#1557446) with sequence similarity to human TNF was discovered in a commercial EST database. Analysis of the 530 nucleotide sequence of the partial cDNA indicated that it encoded an open reading frame for a novel member of the TNF superfamily and was named TL5. The predicted partial protein encoded by this cDNA is 147 amino acids long. This cDNA sequence was used to identify further ESTs which might encode the 5' end of the TL5 cDNA. One such EST encoded a complete open reading frame of 285 amino acids from a cDNA of 1093 nucleotides. The deduced protein encodes a type II membrane protein with a 46 amino acid cytoplasmic domain, an approximately 21 amino acid hydrophobic transmembrane spanning region, followed by a 218 amino acid extracellular domain which shares significant sequence identity with members of the TNF family and presumably encodes the receptor binding portion of the molecule.

A portion of the 3' untranslated sequence of the TL5 cDNA was identical to a human STS DNA sequence (Sequence tagged site) generated from the primer pair SHGC-36171. This fragment has been localized to chromosome 13, which is, therefore, where the gene for TL5 resides.

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

Annex to the description

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: SmithKline Beecham Corporation

(ii) TITLE OF THE INVENTION: A TNF homologue, TL5

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: SmithKline Beecham, Corporate Intellectual Property
(B) STREET: Two New Horizons Court
(C) CITY: Brentford
(D) STATE: Middlesex
(E) COUNTRY: United Kingdom
(F) ZIP: TW8 9EP

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Diskette
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: DOS
(D) SOFTWARE: FastSEQ for Windows Version 2.0

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: TO BE ASSIGNED
(B) FILING DATE: 03-DEC-1997
(C) CLASSIFICATION: UNKNOWN

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 60/041,797
(B) FILING DATE: 02-APR-1997

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: CONNELL, Anthony Christopher
(B) REGISTRATION NUMBER: 5630
(C) REFERENCE/DOCKET NUMBER: GH50016

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: +44 127 964 4395

(B) TELEFAX: +44 181 975 6294
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1093 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
CACGAGAAAA TTCAGGATAA CTCTCCTGAG GGGTGAGCCA AGCCCTGCCA TGTAGTGCAC     60
GCAGGACATC AACAAACACA GATAACAGGA AATGATCCAT TCCCTGTGGT CACTTATTCT    120
AAAGGCCCCA ACCTTCAAAG TTCAAGTAGT GATATGGATG ACTCCACAGA AAGGGAGCAG    180
TCACGCCTTA CTTCTTGCCT TAAGAAAAGA GAAGAAATGA AACTGAAGGA GTGTGTTTCC    240
ATCCTCCCAC GGAAGGAAAG CCCCTCTGTC CGATCCTCCA AAGACGGAAA GCTGCTGGCT    300
GCAACCTTGC TGCTGGCACT GCTGTCTTGC TGCCTCACGG TGGTGTCTTT CTACCAGGTG    360
GCCGCCCTGC AAGGGGACCT GGCCAGCCTC CGGGCAGAGC TGCAGGGCCA CCACGCGGAG    420
AAGCTGCCAG CAGGAGCAGG AGCCCCCAAG GCCGGCCTGG AGGAAGCTCC AGCTGTCACC    480
GCGGGACTGA AAATCTTTGA ACCACCAGCT CCAGGAGAAG GCAACTCCAG TCAGAACAGC    540
AGAAATAAGC GTGCCGTTCA GGGTCCAGAA GAAACAGTCA CTCAAGACTG CTTGCAACTG    600
ATTGCAGACA GTGAAACACC AACTATACAA AAAGGATCTT ACACATTTGT TCCATGGCTT    660
CTCAGCTTTA AAAGGGGAAG TGCCCTAGAA GAAAAAGAGA ATAAAATATT GGTCAAAGAA    720
ACTGGTTACT TTTTTATATA TGGTCAGGTT TTATATACTG ATAAGACCTA CGCCATGGGA    780
CATCTAATTC AGAGGAAGAA GGTCCATGTC TTTGGGGATG AATTGAGTCT GGTGACTTTG    840
TTTCGATGTA TTCAAAATAT GCCTGAAACA CTACCCAATA ATTCCTGCTA TTCAGCTGGC    900
ATTGCAAAAC TGGAAGAAGG AGATGAACTC CAACTTGCAA TACCAAGAGA AAATGCACAA    960
ATATCACTGG ATGGAGATGT CACATTTTTT GGTGCATTGA AACTGCTGTG ACCTACTTAC   1020
ACCATGTCTG TAGCTATTTT CCTCCCTTTC TCTGTACCTC TAAGAAGAAA GAATCTAACT   1080
GAAAATACCA AAA                                                      1093
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 285 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Asp Asp Ser Thr Glu Arg Glu Gln Ser Arg Leu Thr Ser Cys Leu
 1               5                  10                  15
Lys Lys Arg Glu Glu Met Lys Leu Lys Glu Cys Val Ser Ile Leu Pro
            20                  25                  30
Arg Lys Glu Ser Pro Ser Val Arg Ser Ser Lys Asp Gly Lys Leu Leu
        35                  40                  45
Ala Ala Thr Leu Leu Leu Ala Leu Leu Ser Cys Cys Leu Thr Val Val
    50                  55                  60
Ser Phe Tyr Gln Val Ala Ala Leu Gln Gly Asp Leu Ala Ser Leu Arg
65                  70                  75                  80
Ala Glu Leu Gln Gly His His Ala Glu Lys Leu Pro Ala Gly Ala Gly
                85                  90                  95
Ala Pro Lys Ala Gly Leu Glu Glu Ala Pro Ala Val Thr Ala Gly Leu
            100                 105                 110
Lys Ile Phe Glu Pro Pro Ala Pro Gly Glu Gly Asn Ser Ser Gln Asn
        115                 120                 125
Ser Arg Asn Lys Arg Ala Val Gln Gly Pro Glu Glu Thr Val Thr Gln
    130                 135                 140
Asp Cys Leu Gln Leu Ile Ala Asp Ser Glu Thr Pro Thr Ile Gln Lys
145                 150                 155                 160
Gly Ser Tyr Thr Phe Val Pro Trp Leu Leu Ser Phe Lys Arg Gly Ser
                165                 170                 175
Ala Leu Glu Glu Lys Glu Asn Lys Ile Leu Val Lys Glu Thr Gly Tyr
            180                 185                 190
Phe Phe Ile Tyr Gly Gln Val Leu Tyr Thr Asp Lys Thr Tyr Ala Met
        195                 200                 205
Gly His Leu Ile Gln Arg Lys Lys Val His Val Phe Gly Asp Glu Leu
    210                 215                 220
Ser Leu Val Thr Leu Phe Arg Cys Ile Gln Asn Met Pro Glu Thr Leu
225                 230                 235                 240
Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile Ala Lys Leu Glu Glu Gly
                245                 250                 255
Asp Glu Leu Gln Leu Ala Ile Pro Arg Glu Asn Ala Gln Ile Ser Leu
            260                 265                 270
Asp Gly Asp Val Thr Phe Phe Gly Ala Leu Lys Leu Leu
        275                 280                 285
```

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 530 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
GGGAGAAGGC AACTCCAGTC AGAACAGCAG AAATAAGCGT GCCGTTCAGG GTCCAGAAGA    60
AACAGGATCT TACGAGACAT TTGTTCCATG GCTTCTCAGC TTTAAAAGGG GAAGTGCCCT   120
AGAAGAAAAA GAGAATAAAA TATTGGTCAA AGAAACTGGT TACTTTTTTA TATATGGTCA   180
GGTTTTATAT ACTGATAAGA CCTACGCCAT GGGACATCTA ATTCAGAGGA AGAAGGTCCA   240
TGTCTTTGGG GATGAATTGA GTCTGGTGAC TTTGTTTCGA TGTATTCAAA ATATGCCTGA   300
AACACTACCC AATAATTCCT GCTATTCAGC TGGCATTGCA AAACTGGAAG AAGGAGATGA   360
ACTCCAACTT GCAATACCAA GAGAAAATGC ACAAATATCA CTGGATGGAG ATGTCACATT   420
TTTTGGTGCA TTGAAACTGC TGTGACCTAC TTACACCATG TCTGTAGCTA TTTTCCTCCC   480
TTTCTCTGTA CCTCTAAGAA GAAAGAATCT AACTGAAAAT ACCAAAAAAA              530
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 147 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Gly Glu Gly Asn Ser Ser Gln Asn Ser Arg Asn Lys Arg Ala Val Gln
 1               5                  10                  15
Gly Pro Glu Glu Thr Gly Ser Tyr Glu Thr Phe Val Pro Trp Leu Leu
            20                  25                  30
Ser Phe Lys Arg Gly Ser Ala Leu Glu Glu Lys Glu Asn Lys Ile Leu
        35                  40                  45
Val Lys Glu Thr Gly Tyr Phe Phe Ile Tyr Gly Gln Val Leu Tyr Thr
    50                  55                  60
Asp Lys Thr Tyr Ala Met Gly His Leu Ile Gln Arg Lys Lys Val His
65                  70                  75                  80
```

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Val Phe Gly Asp Glu Leu Ser Leu Val Thr Leu Phe Arg Cys Ile Gln
                85                  90                  95
Asn Met Pro Glu Thr Leu Pro Asn Asn Ser Cys Tyr Ser Ala Gly Ile
            100                 105                 110
Ala Lys Leu Glu Glu Gly Asp Glu Leu Gln Leu Ala Ile Pro Arg Glu
        115                 120                 125
Asn Ala Gln Ile Ser Leu Asp Gly Asp Val Thr Phe Phe Gly Ala Leu
    130                 135                 140
Lys Leu Leu
145
```

Claims

1. An isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the TL5 polypeptide of SEQ ID NO:2; or a nucleotide sequence complementary to said isolated polynucleotide.

2. The polynucleotide of claim 1 wherein said polynucleotide comprises the nucleotide sequence contained in SEQ ID NO:1 encoding the TL5 polypeptide of SEQ ID NO2.

3. The polynucleotide of claim 1 wherein said polynucleotide comprises a nucleotide sequence that is at least 80% identical to that of SEQ ID NO: 1 over its entire length.

4. The polynucleotide of claim 3 which is polynucleotide of SEQ ID NO: 1.

5. The polynucleotide of claim 1 which is DNA or RNA.

6. A DNA or RNA molecule comprising an expression system, wherein said expression system is capable of producing a TL5 polypeptide comprising an amino acid sequence, which has at least 80% identity with the polypeptide of SEQ ID NO:2 when said expression system is present in a compatible host cell.

7. A host cell comprising the expression system of claim 6.

8. A process for producing a TL5 polypeptide comprising culturing a host of claim 7 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture.

9. A process for producing a cell which produces a TL5 polypeptide thereof comprising transforming or transfecting a host cell with the expression system of claim 6 such that the host cell, under appropriate culture conditions, produces a TL5 polypeptide.

10. A TL5 polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence of SEQ ID NO:2 over its entire length.

11. The polypeptide of claim 10 which comprises the amino acid sequence of SEQ ID NO:2.

12. An antibody immunospecific for the TL5 polypeptide of claim 10.

13. A method for the treatment of a subject in need of enhanced activity or expression of TL5 polypeptide of claim 10 comprising:

    (a) administering to the subject a therapeutically effective amount of an agonist to said polypeptide; and/or
    (b) providing to the subject an isolated polynucleotide comprising a nucleotide sequence that has at least 80%

identity to a nucleotide sequence encoding the TL5 polypeptide of SEQ ID NO:2 over its entire length; or a nucleotide sequence complementary to said nucleotide sequence in a form so as to effect production of said polypeptide activity *in vivo*.

14. A method for the treatment of a subject having need to inhibit activity or expression of TL5 polypeptide of claim 10 comprising:

(a) administering to the subject a therapeutically effective amount of an antagonist to said polypeptide; and/or
(b) administering to the subject a nucleic acid molecule that inhibits the expression of the nucleotide sequence encoding said polypeptide; and/or
(c) administering to the subject a therapeutically effective amount of a polypeptide that competes with said polypeptide for its ligand, substrate , or receptor.

15. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of TL5 polypeptide of claim 10 in a subject comprising:

(a) determining the presence or absence of a mutation in the nucleotide sequence encoding said TL5 polypeptide in the genome of said subject; and/or
(b) analyzing for the presence or amount of the TL5 polypeptide expression in a sample derived from said subject.

16. A method for identifying compounds which inhibit (antagonize) or agonize the TL5 polypeptide of claim 10 which comprises:

(a) contacting a candidate compound with cells which express the TL5 polypeptide (or cell membrane expressing TL5 polypeptide) or respond to TL5 polypeptide; and
(b) observing the binding, or stimulation or inhibition of a functional response; or comparing the ability of the cells (or cell membrane) which were contacted with the candidate compounds with the same cells which were not contacted for TL5 polypeptide activity.

17. An agonist identified by the method of claim 16.

18. An antagonist identified by the method of claim 16.

19. A recombinant host cell produced by a method of Claim 9 or a membrane thereof expressing a TL5 polypeptide.

European Patent Office

PARTIAL EUROPEAN SEARCH REPORT

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

Application Number EP 98 30 2526

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
E	WO 98 18921 A (HUMAN GENOME SCIENCES, INC.) 7 May 1998 * the whole document especially sequences ID 1 and 2, claims, examples, pages 50-55* ---	1-19	C12N15/19 C07K14/52 C12N5/10 A61K38/19 C07K14/705 C07K16/28
E	WO 98 27114 A (SCHERING CORPORATION) 25 June 1998 * The whole document especially sequences ID 3 and 4 * * claims * ---	1-19	C07K16/24 C12Q1/68 G01N33/68
D,X	L. HILLIER ET AL: "washU-NCI human EST project .zo85e02.s1 Stratagene ovarian cancer (#937219) Homo sapiens cDNA clone 593698 3' " EMBL DATABASE ENTRY HSAA66695, ACCESSION NUMBER AA1166695, 21 December 1996, XP002072308 * abstract * --- -/--	1-5	

TECHNICAL FIELDS SEARCHED (Int.Cl.6)

C07K
C12N
C12Q

INCOMPLETE SEARCH

The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.

Claims searched completely :

Claims searched incompletely :

Claims not searched :

Reason for the limitation of the search:

Although claims 13-14 are directed to a method of treatment of the human/animal body (Article 52(4) EPC), the search has been carried out and based on the alleged effects of the compound/composition.

Place of search	Date of completion of the search	Examiner
THE HAGUE	22 July 1998	Le Cornec, N

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

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PARTIAL EUROPEAN SEARCH REPORT

Application Number
EP 98 30 2526

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
D,X	R.M. MYERS: "Human STS SHGC-36171" EMBL DATABASE ENTRY HSG081, ACCESSION NUMBER G30081, 5 October 1996, XP002072309 * abstract * ---	1-5	
X	. HILLIER ET AL: "The WashU-merck EST project. yf44c01.s2 Homo spiens Cdna clone 129696 3' " EMBL DATABASE ENTRY HS88288, ACCESSION NUMBER R16882, 22 April 1995, XP002072310 * abstract * ---	1-5	
D,A	L. HILLIER ET AL: "The WashU-Merck EST project.ze24d01.r1 Soares fetal heart NbHH19W Homo sapiens cDNA clone 359905 5' similar to TR:G530162 G530162 Tyrosine phosphatase." EMBL DABASE ENTRY HSA62945, ACCESSION NUMBER AA062945, 25 September 1996, XP002072311 * abstract * -----	1-5	TECHNICAL FIELDS SEARCHED (Int.Cl.6)

EPO FORM 1503 03.82 (P04C18)